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Tales of two snails: sexual selection and sexual conflict in *Lymnaea stagnalis* and *Helix aspersa*

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Synopsis Sexual selection and sexual conflict have been shown to play key roles in the evolution of species with separate sexes. Experimental evidence is accumulating that this is also true for simultaneous hermaphrodites. For example, many species of land snails forcefully stab their mating partners with love darts. In the brown garden snail (*Helix aspersa*, now called *Cantareus asperses*), this dart increases sperm storage and paternity, probably via the transfer of an allohormone that inhibits sperm digestion. A recent interspecies comparison of dart-possessing land snails revealed coevolution between darts and spermatophore-receiving organs that is consistent with counteradaptation against an allohormonal manipulation. The great pond snail (*Lymnaea stagnalis*) seems to use a seminal product to manipulate its partner and mates in the male role when enough seminal fluid is available in the prostate gland. Receipt of semen not only initiates egg laying in virgin animals, but also feminizes the mating partner later in life. These increases in the female function have been shown to go at the expense of growth and seminal fluid production of the sperm recipient. Although in *Helix*, and probably also *Lymnaea*, the sperm donor benefits from the induced changes through increased fertilization success, the sperm recipient may experience injury, imposed reallocation of resources, and altered sperm storage. These findings support the existence of sexual conflict in simultaneously hermaphroditic snails, and its importance for the evolution of mating behaviors and reproductive morphologies is discussed.

Introduction

By extending Darwin's theory of sexual selection, research has now firmly established that sexual encounters are usually accompanied by conflicts of interest between partners (for example, Arnqvist and Rowe 2005). Such sexual conflicts arise because traits that are advantageous for one sex can be harmful to the other. As a result, these conflicts can trigger coevolutionary arms races leading to extreme, costly, and sometimes bizarre mating behaviors (for example, Morrow and Arnqvist 2003). In recent years, many studies have focused on sexual conflicts and their consequences (reviewed in Chapman and others 2003; Arnqvist and Rowe 2005). For example, conflicts between the sexes can have severe implications for the evolution of (secondary) sexual characteristics and behaviors and can even lead to speciation.

Most of these previous reports of sexual conflict focused on species with separate sexes. Up to now, hermaphrodites have received relatively little attention in this respect, even though the existence of sexual selection and sexual conflict in hermaphrodites is conceptually challenging. Moreover, because hermaphroditism is common and widespread in the plant and animal kingdom, it is of fundamental importance to understand the selective forces involved. The realization that sexual selection and sexual conflict occur should provide new insights into the radiation and speciation of hermaphrodites, the diversification of hermaphroditic mating behaviors and reproductive structures as well as the underlying genetical, neurophysiological, and developmental mechanisms.

Interestingly, Darwin (1871) believed that sexual selection, which drives sexual conflict, could not act in hermaphroditic organisms, mainly because the sexes are joined within one individual (Darwin 1871). Admittedly, sexual conflict in simultaneous hermaphrodites may seem paradoxical. Nonetheless, it does seem to occur. Here, I want to review several examples of sexual selection and the resulting potential conflicts in simultaneous hermaphrodites. This review will include examples from research on several different hermaphrodites and will especially focus on 2 examples from my own research, the common garden snail *H. aspersa* and the great pond snail *L. stagnalis*. At the same time, these 2 examples nicely illustrate 2 different modes of allohormone transfer, respectively,

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via hypodermic injection and via semen (Koene and Ter Maat 2001, 2002; Koene 2004, 2005b).

Sexual conflict in simultaneous hermaphrodites

Sexual conflict arises when a trait that is beneficial to one mating partner is detrimental to the other. Sperm donors are usually interested in maximizing the number of offspring produced with their sperm, whereas sperm recipients optimize the fitness of their offspring (Chapman and others 2003). These two objectives rarely coincide but are often in conflict, especially when sperm recipients store sperm, mate with different partners, and have specialized sperm-digesting organs. Under such circumstances sexual selection favors sperm donors that manipulate these processes. In turn, such manipulations can evoke counteradaptations by the sperm recipient. In this way, sexual conflict can drive counteradaptive coevolution in hermaphrodites (Koene and Schulenburg 2005). Such an arms race can potentially affect interactions between mating partners, genital morphology, gametes, seminal products, and may even cause speciation. Recent theoretical modeling indicates that such processes can become more extreme in hermaphroditic species than in species with separate sexes, mainly because within one mating simultaneous hermaphrodites gain paternity (male fitness) which can outweigh the loss in female fitness (Michiels and Koene 2006).

The above suggests that sexual selection and sexual conflict have the potential to drive the evolution of the bizarre mating systems and complex reproductive morphologies found in hermaphrodites (Koene and Schulenburg 2005). One example of an extreme morphology is the incredibly long penis of the land slug *Limax corsicus*. This penis spans several times the body length and is used in an elaborate mating sequence where the partners hang from a mucous thread and intertwine their penes (Baur 1998). Examples of even more bizarre behaviors are found in other land slugs. The banana slug *Ariolimax dolichophallus*—for a still unknown reason—occasionally bites off the penis of its partner at the end of copulation (Leonard and others 2002; Reise and Hutchinson 2002). Slugs of the genus *Deroceras* have rather extensive, glandular penial appendages that they use to apply secretions onto the partner's skin (M. Benke, H. Reise, and J. M. Koene, unpublished data) Another example is the repeated hypodermic insemination in tropical flatworms. This so-called penis fencing can be accompanied by severe skin injury (Michiels and Newman 1998). A different form of hypodermic injection is found in the earthworm *Lumbricus terrestris*. Curiously enough, these simultaneous hermaphrodites stab each other with 40–44 specialized setae during copulation to inject a substance from the setal gland into the partner's skin (Koene and others 2002). As a result of this injection, more sperm are taken up and stored differently in the recipient. An equal amount of sperm ends up in each of the 4 spermathecae, rather than predominantly in only 2 when these setae are absent (Koene and others 2005). Based on the cocoon fertilization process, besides increased sperm numbers, this equal distribution of sperm may increase the fertilization chance for the sperm donor. Finally, the 2 examples that I will review here in detail are dart shooting in *H. aspersa* (and other land snails) and seminal fluid transfer in *L. stagnalis*.

Dart shooting in land snails, with a focus on *H. aspersa*

A spectacular example of a bizarre mating behavior in land snails is the shooting of so-called love darts (for example, Adamo and Chase 1988; Koene and Chase 1998a, Koene and Chase 1998b). Note that the term "shoot" is used loosely because the dart does not actually fly through the air. Rather, this pointed calcareous structure is forcefully stabbed through the skin of the mating partner. Besides being an extremely odd behavior, some species have evolved stunning dart shapes (for example, Fedoseeva 1994; Koene and Muratov 2004; Koene 2005a; Koene and Schulenburg 2005). The function of this dart shooting has bewildered scientists since at least the time of Swammerdamm (1637–1680).

To give a better impression of what actually happens when a dart is shot, I will provide here a brief description of the complete courtship and mating sequence of *H. aspersa* (also referred to as *C. aspersus*). This behavior is mainly controlled by the right mesocerebrum, a brain region that has an evolutionarily conserved function in gastropod mollusks (Koene and others 1999, 2000). During the initial stages of courtship the genital atrium is everted and becomes visible as a gradually increasing white bulge on the right side of the animal's head (Adamo and Chase 1988). During this phase pairs can still separate, but once a dart has been shot they rarely do. Dart shooting marks the end of courtship behavior and is typically performed by both animals, though not at the same time (Adamo and Chase 1988). Upon dart shooting, the dart sac—which produces and stores the dart—is forcefully everted from the genital pore, thereby expelling the dart. On its way out, the dart is covered with mucus from the glands associated with the dart sac (often referred to as the digitiform glands). The general result of dart shooting, which is performed once by each partner, is that

the dart perforates the skin of the mating partner and in nearly half of those cases the dart remains lodged in the partner's skin (Adamo and Chase 1988).

Following dart shooting, the penis is everted and each snail attempts to intromit its partner. Simultaneous intromission is required for successful copulation and is achieved when the penes of both snails are inserted into the partners' vaginal duct (Tompa 1984). At this point, the spermatophore is formed in the epiphallus, flagellum, and penis, and filled with sperm. When the spermatophore is completed, it is transferred into the bursa tract diverticulum of the spermatophore-receiving organ of the partner, after which the snails separate. This whole sequence of events is time consuming; the courtship phase lasts approximately 1 h followed by 7 h of copulation on average (Adamo and Chase 1988).

After transfer, sperm can leave the spermatophore by actively swimming out via the spermatophore's tail (formed by the flagellum). In this way they enter the vaginal duct and have a chance of reaching the sperm storage site, the spermathecae (Lind 1973). The spermatophore and the sperm that are left behind in the diverticulum get transported to the bursa copulatrix, the gametolytic part of the spermatophore-receiving organ, for digestion. As a result of this digestion process, only a very small proportion of the sperm makes it up to the spermathecal sacs, where they are stored prior to being used for the fertilization of eggs (Lind 1973; 0.025%: Rogers and Chase 2001). At this point, it is important to note that sperm can be stored for up to 4 years (Duncan 1975) and snails mate with several partners during a season before they lay eggs (Tompa 1984).

Numerous hypotheses have been proposed for the evolution of dart shooting. (reviewed by Kothbauer 1988; Landolfa 2002). One explanation derives from the fact that the dart is made of calcium, in the form of the calcium carbonate crystal aragonite (Tompa 1980). Because calcium is important for the development of snails (Crowell 1973; Tompa 1980), the dart has been proposed to serve as a nuptial gift of calcium for the production of eggs (Charnov 1979; Leonard 1992). For several reasons, this hypothesis has been refuted. The most important reason being that in *H. aspersa* the dart does not contain enough calcium to significantly contribute to egg production (Koene and Chase 1998a). Additionally, darts are only rarely incorporated by the recipient, instead they mostly fall on the ground after having remained stuck in the partner's skin for several hours (Koene and Chase 1998a). That the main function of the dart cannot depend on permanent lodging of the dart in the recipient is also supported by observations in other dart-possessing species because darts can also be retained by the shooters and can even be used repeatedly in the same or a different copulation (J. M. Koene and S. Chiba unpublished data; B. Reyes-Tur and J. M. Koene unpublished data; Webb 1952; Reyes-Tur and others 2000).

Given that none of the above findings support the nuptial gift hypothesis, 2 other types of explanations remain. In the first type the dart is assumed to represent a sexual signal. For example, the dart might signal the readiness of the shooter to lay eggs, thus making it an attractive partner to donate sperm to. No experimental support was found for this idea (Koene and Chase 1998a). The dart could also have a signaling function that could be used in female choice (Leonard 1992, 2005; Landolfa 2002). Given that calcium is an essential element for snail survival, the calcareous dart could provide a signal about the overall condition of the animal. This idea was previously tested by depriving animals of calcium for 8 months. Despite an increased death rate due to shell failure as a result of the lack of calcium, these animals shot darts normally (Koene and Chase 1998a). Finally, a choice could be based on dart shooting effectiveness (Leonard 1992; Landolfa 2002). The important prediction of this hypothesis is that the dart should be shot consistently by individuals (assuming that shooting ability is heritable). Tests in *H. aspersa* do not support this because dart shooting of individually identified nonvirgin animals in consecutive copulations is unpredictable. A significant number of animals that shot in their first mating session did not shoot in the second session and vice versa (G-test: $N = 29$ snails, $df = 1$, $G = 6.745$, $P < 0.01$; Fig. 1). If the dart were an important and reliable signal, the expected outcome would be that animals either shoot in both matings or not at all.

Previous studies have also reported nonvirgin *H. aspersa* occasionally not shooting their darts (Giusti and Lepri 1980; Adamo and Chase 1990; Koene and Chase 1998a; virgins do not shoot: Chung 1986a). Some other species sometimes skip dart shooting (for example, *Helix lucorum*: Giusti and Lepri 1980; *Arianta arbustorum*: Baur and others 1998), and at least for *A. arbustorum* dart shooting may be an optional component of courtship (Baminger and others 2000). But for *H. aspersa*, Chase and Vaga (2006; see also Chung 1987) came to the conclusion that dart shooting is obligatory. Interestingly, they found animals that did not shoot a dart prior to copulation (18 of 94), but these all had empty dart sacs. Their finding is in agreement with my observations on not shooters. I found that animals that did not shoot a dart did have one, but it was no longer attached to the dart sac. Rather, the dart had been dislodged from the dart sac and transported into the bursa tract after

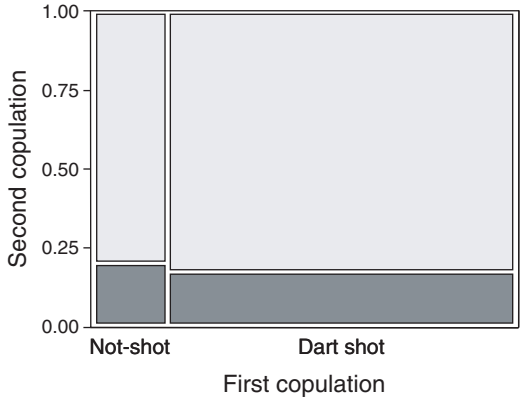

Fig. 1 Contingency table of dart shooting behavior in *H. aspersa* in 2 consecutive copulations. Snails were defined as not shooting if at the time of first penial eversion it had not shot a dart, because dart shooting never occurs once penial eversions begin. All pairs reached successful intromission in both mating trials and thus copulated twice. For these observations, the nonvirgin snails were marked, housed individually at 20–25°C for at least 10 days before the start of the observations, fed every other day, and kept moist. Consecutive mating trials were separated by 2 weeks of sexual isolation to allow for dart regeneration, which takes 5–6 days (Tompa 1982). Light gray, proportion of darts shot in second copulation; Dark gray, proportion of darts not shot in second copulation.

Table 1 The fate of not shot and retracted darts

Position of dart	Not shot	Shot but retracted
Dart sac (detached)	0	2
Genital atrium/copulatory canal	0	2
Bursa tract diverticulum	2	6
Bursa copulatrix	12	2
Expelled	0	3

The positions of not shot ($N = 14$) and retracted darts ($N = 15$) were determined by carefully dissecting individuals after copulation. Darts found in the dart sac were detached from the tubercle (to which the dart is normally attached). Some retracted darts were expelled through the genital pore of the animal during courtship.

copulation, just as retracted darts (Table 1). So, nonvirgin snails that do not shoot a dart prior to copulation either attempted to shoot but failed (and disposed of the dart) or had no dart to shoot. In either case, the fact remains that they do not shoot their dart in a predictable way. Therefore, I conclude that it is rather unlikely that the love dart has a signaling function (see also Adamo and Chase 1996).

The last type of explanation assumes that the dart directly influences either the behavior or the reproductive physiology of the mating partner. It is important to note that this could be achieved either mechanically or chemically. A mechanical effect would be caused by the piercing of the skin itself. A chemical effect would originate from the mucus that covers the dart, which originates from the glands associated with the dart sac. Such an explanation has been suggested in several different forms. For instance, many authors (Dorello 1925; Börnchen 1967; Chung 1986b; Adamo and Chase 1990) have sought to detect an effect of the dart on sexual arousal. Behavioral observations indicate, however, that the receipt of a dart has only a small effect on sexual arousal, as measured by the degree of genital eversion. The result is a slightly shorter courtship (Chung 1986b; Adamo and Chase 1990), which seems a rather small advantage to be gained from such a seemingly costly behavior.

Therefore, in search of a better explanation, the possibility of a physiological effect caused by the mucus that is present on the dart was further explored. Adamo and Chase (1990), again using *H. aspersa*, were able to demonstrate that the mucus that is present on the love dart is introduced into the blood of the partner. This finding indicated that the dart could indeed act as a hypodermic device to deliver a bioactive substance to the interior of the recipient. In a series of physiological experiments I was subsequently able to show that the mucus affects the recipient's female reproductive system (Koene and Chase 1998b). As it turns out, a bioactive component in the mucus causes a reconfiguration of the tract resulting in the closing of the entrance to the duct leading to the bursa copulatrix. This observation suggested that more sperm are enabled to reach the sperm storage organ. Indeed follow-up studies demonstrated that when a dart hits its target, the number of sperm reaching the sperm storage site is higher (Rogers and Chase 2001) and so is paternity (Landolfa and others 2001; Rogers and Chase 2002). Recently, it was also confirmed that it is a component of the mucus on the dart, and not the mechanical stimulation by the dart, that causes this effect (Chase and Blanchard 2006).

These findings indicate that the dart influences the sperm storage process of the partner. The advantage for the shooter of increasing sperm storage in its partner is obvious, especially given that these snails mate several times during a mating season and can store sperm for 4 years. Hence, the dart may have evolved in the competition for the fertilization of eggs. But, while these effects are beneficial for the shooter, receiving a dart may negatively affect the recipient's reproductive fitness. Besides changing the sperm storage process, thus interfering with cryptic female choice, the skin is damaged (especially in species that stab each other repeatedly, see below) and infection rates may be increased.

Taking the above into account, the manipulative effect of the love dart potentially causes a sexual conflict between the shooter and the receiver. In turn, this sexual conflict could lead to countermeasures on the receiver side. Recently this idea was investigated in an interspecies comparison. That study, based on evidence for repeated as well as correlated evolution, revealed that morphological changes in the spermatophore-receiving organs occur in parallel with the evolution of more elaborate darts and dart glands (Koene and Schulenburg 2005). The counteradaptations primarily entail the appearance and subsequent lengthening of a diverticulum, thus increasing the distance sperm need to travel to the spermathecae and thereby offsetting the increased sperm survival caused by more efficient darts. These results support that sexual conflict can drive the coevolutionary arms race between love darts and spermatophore-receiving organs (Koene and Schulenburg 2005). This correlational study provided the first evidence for the existence of theoretically predicted coevolutionary arms races in simultaneous hermaphrodites (see also Schilthuizen 2005).

Besides morphological adaptations to increase the efficiency of the dart, behavioral adaptations can also occur. As I will illustrate below, there is clear evidence that the dart can be used in a range of different ways. At the same time, it will become apparent that there are still a lot of dart-possessing species that warrant close investigation. Within the Helicidae (to which *H. aspersa* also belongs) all investigated species shoot once during courtship, lose their dart in the process, and can make a new dart within a few days. These species all have a single dart with 2–4 perpendicular blades (for example, *A. arbustorum*, *Cepaea nemoralis*, and *Helix pomatia*; Fedoseeva 1994). Within the Hygromiidae the variety of darts is much larger. For example, some have 1 contorted dart with 2 blades like the members of the genus *Leptaxis* (Koene and Muratov 2004) and *Hygromia* (Giusti and Manganelli 1987). Other members of this family have a single dart with up to 7 perpendicular blades (for example, *Monachoides vicinus*: Koene and Schulenburg 2005). Moreover, many hygromiids have 2 darts, like *Trichia* (Schileyko 1978). Unfortunately, despite this wide variety, nothing is known about the way in which the dart is used in this family. Likewise, the dart shooting behavior of most Helminthoglyptidae is unknown. For example, *Helminthoglypta tudiculata* and *Monadenia fidelis* have very different single cone-shaped darts. And one can only begin to imagine what members of the family *Humboldtiana* can do with their 2–8 darts (Thompson and Brewer 2000). The only genus that has been investigated in some detail is *Polymita*. Species of this genus seem to stab their partner repeatedly with a single slender dart that can be reused (Reyes-Tur and others 2000; B. Reyes-Tur and J. M. Koene unpublished data). The same may be true for *Helminthoplypta traski fieldi* (Webb 1952). But, the current champion of repeated stabbing is found within the Bradybaenidae. Most bradybaenids also have a single dart but the exact shape of the dart and blades can vary considerably (Azuma 1995). *Euhadra subnimbosa* has a relatively unspectacular single dart with no real blades, but rather looks lemon-shaped in cross-section. However, it does not lose its dart and uses it repeatedly during courtship at a frequency of approximately 2 stabs per second. As a result, partners stab each other on average over 3000 times (J. M. Koene and S. Chiba, unpublished data), which could be interpreted as a behavioral adaptation to optimize mucus transfer.

Seminal fluid transfer in *L. stagnalis*

The above illustrates an example of sexual conflict in hermaphrodites that mate simultaneously reciprocal. By looking at *L. stagnalis*, I now want to address the question of how such a conflict may work in simultaneous hermaphrodites that do not mate in both roles at the same time. At first sight, mating behavior in the simultaneously hermaphroditic pond snail *L. stagnalis* may seem much less spectacular than the biting, piercing, and stabbing examples described above. But there is more than meets the eye because large amounts of semen are transferred during mating. Besides sperm, the bulk of ejaculate seems to be seminal fluid, originating from the prostate gland. Again, to give a better impression of what actually happens during semen transfer, I will first briefly review the process of reproduction in *L. stagnalis*.

Although *L. stagnalis* is a simultaneous hermaphrodite that can mate in the male and female role, within a copulation one sexual role is performed. Animals seem usually receptive as females and are relatively inactive when copulating in this role (Van Duivenboden and Ter Maat 1985). Hence, most of the resources that are allocated to the female function probably go into egg production rather than female copulatory behavior. Egg laying can be triggered by a transfer from dirty to clean water (Ter Maat and others 1983) and is controlled by a bilateral group of neurons in the cerebral ganglia, the caudo-dorsal cells (CDCs: Ter Maat and others 1986) that release the egg laying hormone (CDCH: Ebberink and others 1985; Geraerts and others 1985; Ter Maat and others 1989; Jiménez and others 2004).

Pond snails are not always motivated to mate in the male role. Male sexual drive increases when individuals have not mated for several days (De Boer and others 1997). The male behavior consists of a fixed sequence of events that starts with shell mounting. The animal crawls to the tip of the shell in a counterclockwise fashion (circling). It then descends to the right side of the partner's shell where it positions itself on the edge. Circling and positioning can be repeated several times. When the right position is found, the partially everted preputium becomes visible. Once the preputium, which carries the penis, is completely everted it probes to find the female opening. After one to several attempts, the penis is intromitted and semen is transferred (De Visser and others 1994; De Boer and others 1997). The seminal fluid is produced by the prostate gland and the increase in size of this gland during sexual isolation motivates the animal to mate in the male role (De Boer and others 1997). This size increase is detected by the brain via a small branch of the penial nerve (De Boer and others 1997). The brain area that receives this information, the anterior lobe, controls male reproductive behavior and is the evolutionary equivalent of the mesocerebrum of *H. aspersa* (Koene and others 2000).

The above indicates that pond snails normally only mate as a male after a period of sexual isolation, when enough seminal fluid is present (Koene and Ter Maat 2005). This increased eagerness to mate after sexual isolation seems to be a common phenomenon in simultaneous hermaphrodites (*Aplysia fasciata*: Ziv and others 1989; *H. aspersa*: Adamo and Chase 1990; *Dugesia polychroa*: Peters and others 1996). In the case of *L. stagnalis*, when both individuals are motivated to mate as males, the individual that has been sexually isolated longest will act as male first; afterward, role alternation can take place so that both individuals get to mate in both roles sequentially (Van Duivenboden and Ter Maat 1985). The occurrence of role alternation *per se* has often been interpreted as evidence for sperm trading, thus solving the conflict between mating partners over sexual roles (for example, Leonard 1991, 2005). Interestingly, in *L. stagnalis* role alternation only takes place within a mating pair when both individuals are motivated to mate in the male role (Koene and Ter Maat 2005). The fact that not-isolated individuals that are inseminated only very rarely show role alternation demonstrates that insemination does not evoke a switch in the sexual role of the sperm recipient. Hence, this finding suggests that these snails will only mate in the male role when enough seminal fluid is present for successful fertilization. From this I conclude that, in general, copulation in *L. stagnalis* is based on unconditional reciprocity, although there may be a conditional component in pairs of isolated snails (Koene and Ter Maat 2005).

The above implies that sex role alternation is entirely driven by the motivation to mate as a male, based on the state of the prostate gland. This makes sense, given that male reproductive investment equals the energetic costs for the hermaphrodite's female reproduction. This was elegantly demonstrated by De Visser and colleagues (1994) via experimental elimination of the male behavior, which resulted in doubled egg production. In the original publication of those results, 2 experimental control groups were lumped together in the statistical analysis. Some have interpreted this as a weakness in the analysis or data, and it has therefore not received the appreciation that this study deserves. As I show here in a reanalysis of the original data, the experimental group differs significantly from both control groups (oneway ANOVA: $F_{2,25} = 9.22$ $P = 0.001$; Post-hoc Tukey: $P < 0.005$; Fig. 2). Hence, this study remains the clearest demonstration to date of the equal distribution of resources between the male and female function, as predicted by theory (Charnov 1979; Greeff and Michiels 1999).

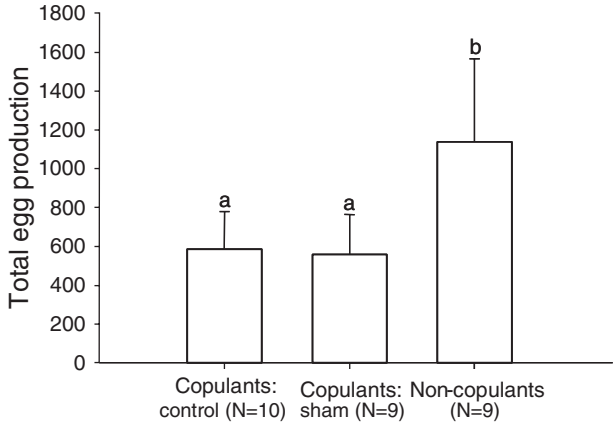

Fig. 2 Reanalysis of the *L. stagnalis* data from De Visser and colleagues (1994). The authors described the methods in detail. In brief, in the experimental group male behavior was eliminated by cutting the nerve between the prostate gland and the central nervous system (noncopulants). The 2 control groups (both copulants) were untreated (copulants: control) and sham operated (copulants: sham). Animals were individually housed and their consumption of the standardized amount of lettuce (39 cm^2) was measured daily. Egg laying was also monitored daily whereas growth and dry weight were measured at the end. The significant difference in egg laying is indicated by different letters. No differences in growth (oneway ANOVA: $F_{2,25} = 0.64$, $P = 0.535$), consumption (oneway ANOVA: $F_{2,25} = 1.74$, $P = 0.196$), and dry weight (oneway ANOVA: $F_{2,25} = 2.01$, $P = 0.155$) were found between the 3 groups.

Given this high male investment, these snails should be prudent with their expensive male reserves. One way to achieve this would be by preferentially inseminating different partners. A recent study revealed that these snails indeed inseminate a new partner even when their prostate gland is partially depleted (J. M. Koene and A. Ter Maat unpublished data). Hence, despite a reduced drive to remate with its original partner (De Boer and others 1997), *Lymnaea* readily mates again, provided that the partner is new (J. M. Koene and A. Ter Maat, unpublished data). This finding indicates that familiarity of the partner is an additional factor that affects male motivation. The rekindled sexual motivation when an unfamiliar partner is encountered has been dubbed the Coolidge effect after an anecdote about President Coolidge and his wife. This phenomenon was first demonstrated in rats but seems to be widespread among promiscuous vertebrates (Fowler and Whalen 1961; Wilson and others 1963; Pizzari and others 2003). Although previously suggested for *Aplysia* (Ziv and others 1989), to the best of my knowledge the *Lymnaea* study represents the first direct evidence for a Coolidge effect in a hermaphrodite.

Understanding these motivation issues also provides insight into the way that this simultaneous hermaphrodite attempts to optimize its male investment. Clearly, the above findings illustrate the importance of the transfer of seminal fluid alongside with the sperm. And it is the seminal fluid, originating from the prostate gland, that makes up the bulk of the ejaculate. This gland produces several bioactive substances that can potentially act as allohormones (Koene and Ter Maat 2001, 2002, 2004; J. M. Koene, A. Ter Maat, and G. T. Nagle, unpublished data) that could be used to manipulate the mating partner. If such a manipulation goes against the recipient's best interests, a sexual conflict can be the result (Koene and Ter Maat 2004; Koene and others 2006). In *L. stagnalis*, as in many hermaphrodites, sexual conflict can occur over the use of sperm by the partner and/or over the allocation of resources in the partner. Both options will be explored in the following.

Conflict over sperm use occurs because digestion of the majority of received sperm is common practice in hermaphrodites. In *L. stagnalis* this sperm digestion takes place in a specialized, gametolytic gland called the bursa copulatrix. Although a large ejaculate is transferred (De Visser and others 1994) only a small proportion of the sperm reaches the sperm storage site (J. M. Koene, K. Montagne-Wajer, and A. Ter Maat, unpublished data), from which these sperm can be used for fertilization for up to 3 months (Cain 1956). Theoretical work has shown that the large investment in the male function probably results from sperm digestion and storage (Greeff and Michiels 1999). What has remained unexplored in *L. stagnalis* is whether animals try to influence the fate of their sperm after donation. Animals could, for instance, increase their fertilization success by inhibiting either sperm digestion or remating in the partner. To achieve this, animals could exploit neurobiological or physiological properties of the female system (sensory trap: Christy 1995; Koene 2005b). For example, an allohormone in the semen may actively manipulate female processes, while having to digest the large ejaculate may prevent remating in itself. Fertilization-enhancing agents are often present in semen (Insects: Simmons 2001; Mammals: Gomendio and others 1998), while there are also examples of inhibited remating when the sperm receiving organ is full (for example, *Pieris rapae crucivora*: Sugawara 1979).

Conflict over resource allocation occurs because simultaneous hermaphrodites can divide their resources in a phenotypically plastic way over growth, the male and female function (Hughes and others 2002; Schärer and others 2003). Although this allows for short-term adjustments in sex allocation in response to environmental factors affecting mating group size and composition, this flexibility can also be disadvantageous. The disadvantage arises because individuals may not agree about the allocation of resources in their mating partners. In turn, this can result in a sexual conflict over resource allocation, in which individuals attempt to manipulate their partner's allocation.

Van Duivenboden (1983) had already demonstrated that the receipt of semen can accelerate the onset of the female function. At the time, this finding was interpreted as a mechanism to initiate the female function at the appropriate time (Van Duivenboden 1983; Insects: Gillott 2002). As a result, the animals may delay selfing and avoid inbreeding (Tsitrone and others 2003). However, a follow-up study that compared mated and unmated virgin snails revealed that the earlier onset of egg laying affects resource allocation, and actually goes at the expense of both body growth and prostate gland development (Koene and Ter Maat 2004). To investigate whether this resource allocation occurs only at the start of egg laying, we subsequently compared animals that were offered one or several mating opportunities. Again, the animals that mated repeatedly at set intervals of 7 days produced more eggs and these repeatedly-grouped snails also developed smaller prostate glands. Hence, mating frequency also influences resource allocation. Moreover, the decrease in prostate gland development suggests that the investment in seminal fluid production is

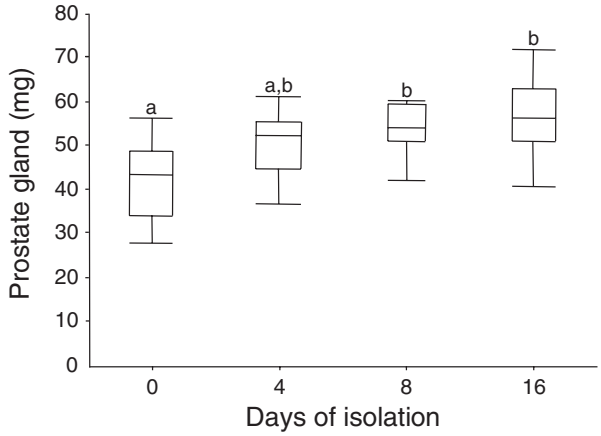

Fig. 3 Effect of sexual isolation on the weight of the prostate gland. Four groups of twelve snails with a shell height of 30–33 mm were isolated for 0, 4, 8, and 16 days. subsequently their body weight was measured and the prostate gland was removed and weighed. The box plots show median, 25th and 75th quartile, and range. The significant differences in gland weight are indicated by different letters (oneway ANOVA: $F_{3,42} = 6.74$, $P = 0.0008$; Post-hoc Student's *t*: $P < 0.005$). No differences were found in body weight between the different groups (oneway ANOVA: $F_{3,42} = 0.78$, $P = 0.513$).

lower in the repeatedly-grouped animals. Important to note here is that this difference in size of the prostate gland cannot be due to copulation because a prostate-replenishing period of 7 days was taken into account before the glands were weighed (De Boer and others 1997; Fig. 3). Hence, receiving semen seems to increase egg laying and thereby actually reduces investment into part of the male function, namely seminal fluid production. The increase in egg production may be mediated by an allohormone that triggers egg laying in the recipient (Koene and others 2006). Obviously, this feminization of the partner is beneficial for the male reproductive success of the sperm donor. But this shift in allocation may conflict with the sperm recipient's interests because it could potentially reduce male reproductive success. In summary, evidence is accumulating for a sexual conflict over resource allocation in *L. stagnalis*, although it remains to be shown that the male function is indeed negatively affected.

Concluding remarks

From the above review, I conclude that in simultaneous hermaphrodites—like in species with separate sexes—sexual conflict can severely impact the evolution of reproductive morphologies and mating behaviors. For dart shooting land snails, coevolution between love darts and spermatophore-receiving organs has been revealed. That this results in a coevolutionary arms race is supported by evidence for both correlated and repeated evolution. But besides morphological adaptations, evidence is accumulating that behavioral adaptations can also occur to optimize mucus transfer via the dart. *E. subnimbosa* seems to represent an extreme case where the partners stab each other a staggering number of times before donating sperm.

Recent experiments with *L. stagnalis* indicate that sexual conflict also occurs in simultaneous hermaphrodites where the sexual roles are performed separately. Repeated mating results in a feminization of the partner and at the same time seems to decrease seminal fluid production. This finding illustrates the tradeoff between the female and male function in this simultaneous hermaphrodite. Moreover, it hints at a conflict over resource allocation between the sperm donor and the sperm recipient, which may be mediated by an allohormone.

Evidently, the 2 species that this review focused on differ in many important aspects of their reproductive habits. For instance, *H. aspersa* donates a spermatophore and mates simultaneously reciprocal in a face-to-face position, whereas *L. stagnalis* donates sperm in seminal fluid and mates unilaterally in a shell-mounting fashion. Whether these differences are responsible for the different manifestations of sexual selection and sexual conflict observed in these species clearly requires a more substantial comparative study (but see Davison and others 2005). The foregoing also illustrated that the mating partner can be influenced by an allohormone that can be transferred via hypodermic injection or semen. Finally, the resulting sexual conflict has the potential of playing a key role in the evolution of reproductive morphology and mating behavior of simultaneous hermaphrodites, and can result in a coevolutionary arms race.

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